50. 49
~~52~~. The method of Claim ~~51~~, wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

Sub C5
53. The method of Claim 44, wherein said microorganism comprises virus.

52. 51
~~54~~. The method of Claim ~~53~~, wherein said virus is selected from the group consisting of ~~comprising~~ hepatitis C virus and simian immunodeficiency virus.

REMARKS

Status of the Application

Claims 1-44 were at issue in the present application and stand rejected.

Amendments to the Specification

The amendments to the specification serve to include the SEQ ID NOs for sequences shown in Figures 6, 20, 21 and 23 and serve to acknowledge government support. In addition, the specification is amended to include the generic terminology for the trademark "Cleavase™" (Applicants note that federal registration has been obtained for this mark). Support for these amendments is provided by the specification at pages 199-305 where the Cleavase™ enzymes are referred to with the appropriate generic terminology following the trademark (*see, e.g.*, page 199, l. 20). Further, these amendments serve to correct typographical errors.

Amendments to the Claims

Claims 1, 19 and 44 have been amended to recite that the cleavage means employed is an enzymatic cleavage means. These amendments are fully supported by the specification which teaches that pages 33-36 and 56-101 and in Examples 10-37 (pages 142-305) which teaches that enzymatic cleavage means (*e.g.*, structure-specific nucleases) are used to cleave nucleic acid substrates which contain cleavage structures (*i.e.*, areas of secondary structure formed by intra-strand hydrogen bonding) to produce a unique or characteristic set of cleavage products. The specification teaches that a variety of structure-specific nucleases can

be employed in the methods of the present invention, including the Cleavase® enzymes (*e.g.*, the Cleavase® BN nuclease), DNA polymerases having 5' nuclease activity (*e.g.*, *Thermus aquaticus* DNA polymerase, *Thermus flavus* DNA polymerase, *Thermus thermophilus* DNA polymerase and *E. coli* DNA polymerase I), *E. coli* Exo III, and eukaryotic structure-specific endonucleases (*e.g.*, the yeast RAD2 protein and the RAD1/RAD10 complex and murine FEN-1 endonucleases) and the calf 5' to 3' exonuclease. (Specification at p. 33, l. 29 - p. 34 l. 24 as well as Examples 10-37). In addition, the specification teaches that the structure-specific nucleases may be provided in solutions comprising manganese. Therefore, these amendments introduce no new matter. In view of the amendments to Claims 1 and 19, Claims 6 and 30 have been canceled. Claim 1 has also been amended to recite that cleavage products generated by cleavage of a test substrate are compared to the products produced by cleavage of a control or reference substrate. This amendment is fully supported by the specification (see e.g., page 16, ll. 24-30) and introduces no new matter.

Claim 19 has also been amended to recite that following cleavage of the extracted microorganismal nucleic acid a plurality of cleavage products is produced. This amendment is supported by the specification in Examples 34-36 and the accompanying figures which show that a plurality of cleavage products is produced by cleavage of bacterial and viral nucleic acid substrates. Therefore, no new matter is introduced by this amendment.

Claims 4 and 32 have been amended to include the generic terminology for the trademark "Cleavase™ BN". As discussed above, the specification provides support for these amendments at pages 199-305 where the Cleavase™ enzymes, including the Cleavase™ BN enzyme, are referred to with the appropriate generic terminology following the trademark (*see, e.g.*, page 199, l. 20). Therefore, these amendments introduce no new matter.

Dependent Claims 45-54 have been added. These claims are fully supported by the specification and introduce no new matter.

The Examiner has made a number of remarks and rejections. For clarity, the rejections at issue are set forth below:

(1) The Examiner requests that the brief description of the drawings for Figures 6, 20-21 and 23-24 be amended to recite the appropriate SEQ ID NO for the sequences shown in these figures.

(2) Claim 1 is rejected under 35 U.S.C. § 112 as being indefinite.

(3) Claims 1-44 are rejected under U.S.C. § 101 as being improper process claims.

(4) Claims 1-44 are rejected under U.S.C. § 103(a) as being unpatentable over Lyamichev *et al.* (Science 260:778-783) in view of Young (U.S. Patent No. 5,422,242), Seela and Röling (Nuc. Acids Res. 20:55-61) and Young *et al.* (J. Clin. Microbiol. 31:882-886).

Applicants believe that the above amendments and the following remarks overcome the Examiner's rejection of the claims. These remarks are presented in the same order as they appear above.

(1) Addition Of SEQ ID NOs To Description Of The Drawings

The specification has been amended to indicate the SEQ ID NOs of the nucleotide sequences shown in Figures 6, 20, 21A-D and 23. No amendment was made concerning Figure 24 as this figure does not show any nucleotide sequences; the "A," "T" and "A/T" shown above the lanes in Figure 24 indicate which type of bead (either a bead comprising the A-hairpin, the T-hairpin or both in linear form) was present in the reaction.

(2) Claim 1 Is Definite

Claim 1 was rejected as being indefinite under 35 U.S.C. § 112 for failure to "set forth **all** steps involved in the method/process . . ." (Office Action at p. 2; emphasis added). There is no requirement that a claim set forth **all** steps in a method/process. Form ¶ 7.34.06, Use Claims, states that a claim is to be rejected form indefiniteness when "the claim does not set forth **any** steps involved in the method/process . . ." [MPEP 706.03(d); emphasis added]. Indeed, the Examiner appears to acknowledge this as he states that a "claim is indefinite where it merely recites a use without **any** active, positive steps delimiting how this use is actually practiced." (Office Action at p. 2; emphasis added). Amended Claim 1 sets forth the following active, positive steps: 1) providing an enzymatic cleavage means and a nucleic acid substrate containing microbial sequences; 2) treating the nucleic acid substrate under conditions such that at least one cleavage structure is formed; and 3) cleaving the cleavage structure with the enzymatic cleavage means to generate cleavage products.

Claim 1 defines an operative process which includes the essential or critical steps of the process (*i.e.*, the formation of cleavage structures in a nucleic acid substrate derived from a microorganism and the cleavage of these structures to form cleavage products). The

specification provides specific definitions for the terms "cleavage means," "cleavage structure" and "cleavage products" at pages 33-34. The specification teaches conditions that permit the formation of cleavage structures in a nucleic acid substrate (*see* pages 56-57 as well as Examples 10-37).

As Claim 1 sets forth active, positive steps, the rejection of this Claim under 35 U.S.C. § 112 is improper. Applicants request that this rejection be withdrawn.

(3) Claims 1-44 Are Proper Process Claims

Claims 1-44 were rejected under 35 U.S.C. § 101 for reciting "a use, without setting forth **all** steps involved in the process . . ." (Office Action, p. 2; emphasis added). This rejection appears to be a misquote of Form ¶ 7.34.06, Use Claims, which states that a claim is to be rejected under 35 U.S.C. § 101 when a claim recites "a use, without setting forth **any** steps in the process . . ." [MPEP 706.03(d); emphasis added]. Independent Claims 1, 19 and 44 all set forth the active, positive steps involved in the claimed methods (all other claims are dependent upon these three independent claims). The active steps of Claim 1 were discussed above. Amended Claim 19 sets forth the following active, positive steps: 1) extracting nucleic acid from a sample suspected of containing at least one microorganism; 2) contacting the extracted nucleic acid with an enzymatic cleavage means under conditions such that cleavage structures are formed in the extracted nucleic acid and the cleavage means cleavages these structures to generate cleavage products. Amended Claim 44 sets forth the following active, positive steps: 1) providing an enzymatic cleavage means in a solution containing manganese and a nucleic acid substrate containing microbial gene sequence; 2) treating the nucleic acid substrate with increased temperature to render the substrate substantially single-stranded; 3) reducing the temperature such that cleavage structures are formed; 4) cleaving the cleavage structures with the cleavage means to generate cleavage products; and 5) detecting the cleavage products.

As Claims 1-44 provide active, positive steps, the rejection of these claims under 35 U.S.C. § 101 and/or § 112 is improper. Applicants request that this rejection be withdrawn.

(4) Claims 1-44 Are Unobvious

Claims 1-44 are rejected under U.S.C. § 103(a) as being unpatentable over Lyamichev *et al.* in view of Young, Seela and Röling, and Young *et al.* Applicants disagree. No motivation to combine these references to arrive at the claimed invention is provided by these references and the Examiner has failed to explain why one of ordinary skill in the art at the time the invention was made would have been motivated to combine these references.[1] Therefore, no *prima facia* case of obviousness has been made. Assuming *arguendo* that a *prima facia* case is established, the combination of these references does not result in the claimed invention.

The focus of the Lyamichev *et al.* reference is the use of the 5' nuclease activity of certain DNA polymerases to cleave nucleic acids in a sequence-independent manner; this method requires the inter-strand hybridization of at least two nucleic acids. The abstract of this reference states that "[e]ssentially any linear single-stranded nucleic acid can be targeted for specific cleavage by the 5' nuclease activity of DNA polymerase through hybridization with an oligonucleotide that converts the desired cleavage site into a substrate." While Lyamichev *et al.* teach that the 5' nuclease activity of DNA polymerases may be used to cleave a hairpin structure in the absence of an annealed oligonucleotide (*i.e.*, a primer), this reference further teaches that primer-independent cleavage of hairpin structures is much less efficient than primer-directed cleavage (see pages 779-780). Therefore, this reference can be said to teach away from a method in which primers are not employed for the cleavage of intra-strand structures such as hairpins.

Lyamichev *et al.* fails to teach that the 5' nuclease activity of DNA polymerases activity may be used to detect sequence variation between nucleic acids by cleavage of **intra**-strand secondary structures (*i.e.*, the cleavage structures of the claimed invention). Rather

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[1] "[W]hen the PTO asserts that there is an explicit or implicit teaching or suggestion in the prior art, it must indicate where such a teaching or suggestion appears." *In re Rijckaert*, 9 F.3d 1531, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). See also the M.P.E.P at 706.02(j).

Lyamichev *et al.* teach the detection of sequence variation by cleavage of one strand in a structure formed by **inter**-strand annealing:

> The 5' nucleases of thermostable DNAPs may also be useful in probing for sequences or sequence variants in nucleic acid preparations. For example, the presence of a particular DNA might be detected by using that molecule as the template strand for **cleavage of an added substrate probe** . . ." (pp. 782-783; emphasis added).

Contrary to the Examiner's assertion on page 4 of the Office Action, Lyamichev *et al.* do not teach the intra-strand cleavage of RNA as required by the claims. Cleavage of RNA substrates was shown to be dependent upon the presence of a pilot oligonucleotide (*see* Fig. 6C of Lyamichev *et al.* on p. 781 and the corresponding discussion on p. 782). Therefore, Lyamichev *et al.* fail to teach: 1) a method for the detection of sequence variation between nucleic acids by cleavage of intra-strand secondary structures and 2) cleavage of RNA substrates in the absence of an additional oligonucleotide. Further, as the Examiner has admitted on page 5 of the Office Action, this reference fails to teach: 3) a method for identifying strains of microorganisms; 4) a method for the identification of microorganisms wherein cleavage products from a test sample are compared to cleavage products generated from a reference microorganism; 5) a method wherein the substrate is generated in a PCR using a nucleotide analog.

The Examiner states that "Lyamichev *et al.* taught that this method can be used to optimize allele-specific PCR" (Office Action at page 5). Lyamichev *et al.* suggested a method of optimizing allele-specific PCR wherein the 5' nuclease activity of a DNA polymerase is employed in a PCR to selectively cleave unwanted templates that are annealed to a primer having an unpaired 3' end (Lyamichev *et al.* at p. 782, right-hand column); this is cleavage of a cleavage structure formed by inter-strand hybridization of two separate nucleic acids. This method of optimizing allele-specific PCR has nothing to do with the claimed invention which requires the cleavage of intra-strand secondary structures which are formed in the absence of an annealed primer. Applicants further note that the claimed methods do not require that DNA polymerization occur; in contrast allele-specific PCR, like all PCRs, requires DNA synthesis.

The secondary references cited by the Examiner fail to remedy the deficiencies of the primary reference. Young *et al.* teach primers which are used to amplify (in a PCR) regions

of the 16S rRNA genes of *Mycobacterium* and probes capable of hybridizing to the amplified region; species-specific probes are provided which permit the identification of a particular species of *Mycobacterium* in a sample. Seela and Röling teach the use of 7-deazapurine nucleotides in a PCR. Young *et al.* teach a RT-PCR assay for the detection of hepatitis C virus. None of these secondary references teach or suggest a method of identification of microorganisms by cleavage of intra-strand cleavage structures in a nucleic acid substrate derived from a microorganism to generate a characteristic set of cleavage products.

The Examiner states that those "of ordinary skill in the art would have been motivated to combine the teachings of Lyamichev et al. with Young, Seela and Roling, and Young et al. to produce a method that could be used to optimize allele-specific PCR . . ." (Office Action at p. 8). Applicants disagree. As stated above, Lyamichev *et al.* do not teach methods relevant the claimed methods for the detection of sequence variation between nucleic acids by cleavage of intra-strand secondary structures which are formed in the absence of an annealed primer. Therefore, no basis exists for combining the secondary references with Lyamichev *et al.* to arrive at the claimed invention.

As the Examiner has failed to cite a reference or a combination of references which teaches or suggests the claimed invention, no *prima facie* case of obviousness has been made and therefore, the rejection of Claims 1-44 under 35 U.S.C. § 103 is improper. Applicants request that this rejection be withdrawn.

(5) Request For Entry Of References Supplied In The Accompanying Information Disclosure Statement

Applicants have submitted an Information Disclosure Statement (IDS) and Form PTO-1449 with this response. Applicant respectfully request entry of the references cited in this IDS.

CONCLUSION

Applicants respectfully request that the above amendments be entered. For the reasons set forth above, it is respectfully submitted that Applicants' claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned collect at (415) 299-8120.

Dated: June 6, 1997

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APPENDIX A

All claims now pending are reproduced below as they will appear following entry of the present amendment, for the convenience of the Examiner.

1. A method, comprising:

a) providing:

i) an enzymatic cleavage means;

ii) a nucleic acid substrate containing sequences derived from one or more microorganism; and

iii) control cleavage products produced by cleavage of a reference sequence derived from a microorganism;

b) treating said nucleic acid substrate under conditions such that said substrate forms one or more cleavage structures;

c) reacting said cleavage means with said cleavage structures so that one or more cleavage products are produced; and

d) comparing said test cleavage products to said control cleavage products.

3. The method of Claim 1, wherein said enzymatic cleavage means is a nuclease.

4. The method of Claim 3, wherein said nuclease is selected from the group consisting of Cleavase™ BN nuclease, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

5. The method of Claim 1, wherein said nucleic acid substrate comprises a nucleotide analog.

6. The method of Claim 5, wherein said nucleotide analog is selected from the group comprising 7-deaza-dATP, 7-deaza-dGTP and dUTP.

7. The method of Claim 1, wherein said nucleic acid of step (a) is substantially single-stranded.

8. The method of Claim 1, wherein said nucleic acid is RNA.

9. The method of Claim 1, wherein said nucleic acid is DNA.

10. The method of Claim 1, wherein said nucleic acid of step (a) is double stranded.

11. The method of Claim 10, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

12. The method of Claim 11, wherein said double-stranded nucleic acid is rendered substantially single-stranded by increased temperature.

13. The method of Claim 1, further comprising the step of detecting said one or more cleavage products.

14. The method of Claim 1 wherein said microorganism comprises bacteria.

15. The method of Claim 14 wherein said bacteria are selected from the group comprising members of the genera *Campylobacter, Escherichia, Mycobacterium, Salmonella, Shigella* and *Staphylococcus*.

16. The method of Claim 15 wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

17. The method of Claim 1 wherein said microorganism comprises virus.

18. The method of Claim 17 wherein said virus is selected from the group comprising hepatitis C virus and simian immunodeficiency virus.

19. A method for detecting and identifying strains of microorganisms, comprising:

a) extracting nucleic acid from a sample suspected of containing one or more microorganisms; and

b) contacting said extracted nucleic acid with an enzymatic cleavage means under conditions such that said extracted nucleic acid forms one or more secondary structures, and said cleavage means cleaves said secondary structures to produce a plurality of cleavage products.

20. The method of Claim 19, further comprising the step of separating said cleavage products.

21. The method of Claim 19, further comprising the step of detecting said cleavage products.

22. The method of Claim 21, further comprising comparing said detected cleavage products generated from cleavage of said extracted nucleic acid isolated from said sample with separated cleavage products generated by cleavage of nucleic acids derived from one or more reference microorganisms.

23. The method of Claim 19 further comprising the step of isolating a polymorphic locus from said extracted nucleic acid after the extraction of step a), to generate a nucleic acid substrate wherein said substrate is contacted with the cleavage means of step b.

24. The method of Claim 23 wherein said isolation of a polymorphic locus is accomplished by polymerase chain reaction amplification.

25. The method of Claim 24, wherein said polymerase chain reaction is conducted in the presence of a nucleotide analog.

26. The method of Claim 25, wherein said nucleotide analog is selected from the group comprising 7-deaza-dATP, 7-deaza-dGTP and dUTP.

27. The method of Claim 24 wherein said polymerase chain reaction amplification employs oligonucleotide primers matching or complementary to consensus gene sequences derived from said polymorphic locus.

28. The method of Claim 23 wherein said polymorphic locus comprises a ribosomal RNA gene.

29. The method of Claim 28, wherein said ribosomal RNA gene is a 16S ribosomal RNA gene.

31. The method of Claim 19, wherein said enzymatic cleavage means is a nuclease.

32. The method of Claim 31, wherein said nuclease is selected from the group consisting of Cleavase™ BN nuclease, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

33. The method of Claim 19, wherein said nucleic acid of step (a) is substantially single-stranded.

34. The method of Claim 19, wherein said nucleic acid is RNA.

35. The method of Claim 19, wherein said nucleic acid is DNA.

36. The method of Claim 19, wherein said nucleic acid of step (a) is double stranded.

37. The method of Claim 36, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

38. The method of Claim 37, wherein said double-stranded nucleic acid is rendered substantially single-stranded by increased temperature.

39. The method of Claim 19 wherein said microorganism comprises bacteria.

40. The method of Claim 39 wherein said bacteria are selected from the group comprising members of the genera *Campylobacter, Escherichia, Mycobacterium, Salmonella, Shigella* and *Staphylococcus*.

41. The method of Claim 40 wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

42. The method of Claim 19 wherein said microorganism comprises virus.

43. The method of Claim 42 wherein said virus is selected from the group comprising hepatitis C virus and simian immunodeficiency virus.

44. A method, comprising:

a) providing:

i) an enzymatic cleavage means in a solution comprising manganese; and

ii) nucleic acid substrate containing microbial gene sequences;

b) treating said nucleic acid substrate with increased temperature such that said substrate is substantially single-stranded;

c) reducing said temperature under conditions such that said single-stranded substrate forms one or more cleavage structures;

d) reacting said cleavage means with said cleavage structures so that one or more cleavage products are produced; and

e) detecting said one or more cleavage products.

45. The method of Claim 44, wherein said enzymatic cleavage means is a nuclease.

46. The method of Claim 45, wherein said nuclease is selected from the group consisting of Cleavase™ BN nuclease, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

47. The method of Claim 44, wherein said nucleic acid substrate comprises a nucleotide analog.

48. The method of Claim 47, wherein said nucleotide analog is selected from the group comprising 7-deaza-dATP, 7-deaza-dGTP and dUTP.

49. The method of Claim 44, wherein said nucleic acid is selected from the group consisting of RNA, double stranded DNA and single stranded DNA.

50. The method of Claim 44, wherein said microorganism comprises bacteria.

51. The method of Claim 50, wherein said bacteria are selected from the group comprising members of the genera *Campylobacter, Escherichia, Mycobacterium, Salmonella, Shigella* and *Staphylococcus*.

52. The method of Claim 51, wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

53. The method of Claim 44, wherein said microorganism comprises virus.

54. The method of Claim 53, wherein said virus is selected from the group comprising hepatitis C virus and simian immunodeficiency virus.